

# Range expansion for the Critically Endangered poison-dart frog *Leucostethus bilsa* Vigle et al., 2020 (Amphibia, Dendrobatidae), demonstrating the importance of small forest reserves in the Ecuadorian Chocó

S. Holden Jones<sup>1</sup>, Daniela Franco-Mena<sup>2</sup>, Darwin Zambrano Cantos<sup>3</sup>, Julio César Loor Vélez<sup>3</sup>, Carlos Aulestia<sup>3</sup>, Rakan A. Zahawi<sup>1,4</sup>, Juan M. Guayasamin<sup>2</sup>

<sup>1</sup> School of Life Sciences, University of Hawai'i at Mānoa, Honolulu, Hawaii, USA

<sup>2</sup> Laboratorio de Biología Evolutiva, Instituto Biósfera, Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito (USFQ), Quito, Ecuador

<sup>3</sup> Fundación para la Conservación de los Andes Tropicales (FCAT), Quito, Ecuador

<sup>4</sup> Charles Darwin Foundation, Puerto Ayora, Galapagos, Ecuador

Corresponding author: S. Holden Jones (jonessh@hawaii.edu)

**Abstract.** The Ecuadorian Chocó is a global conservation hotspot with high levels of biodiversity and endemism but is threatened by extensive deforestation. Here, we report the discovery of an additional locality for the Critically Endangered poison-dart frog *Leucostethus bilsa* (Amphibia, Dendrobatidae) in a privately protected forest reserve in Esmeraldas, Ecuador. This may represent a distinct IUCN location for the species, thereby downgrading its IUCN threat level to Endangered. This finding increases the extent of occurrence for *L. bilsa* from 0.9 to 4.78 km<sup>2</sup>, and the area of occupancy from 8.0 to 16.0 km<sup>2</sup>, demonstrating the importance of small forest reserves for the conservation of forest-dependent amphibians. Further surveys are warranted to determine whether the species is present in additional forest fragments in the region, the degree of connectivity, if any, between individuals inhabiting different fragments, and whether the species' population is increasing, stable, or in decline.

**Key words.** Amphibians, connectivity, conservation, forest-dependence, fragmentation

**Jones SH, Franco-Mena D, Cantos DZ, Loor Vélez JC, Aulestia C, Zahawi RA, Guayasamin JM** (2024) Range expansion for the Critically Endangered poison-dart frog *Leucostethus bilsa* Vigle et al., 2020 (Amphibia, Dendrobatidae), demonstrating the importance of small forest reserves in the Ecuadorian Chocó. Check List 20 (4): 853–858. <https://doi.org/10.15560/20.4.853>

## INTRODUCTION

Dendrobatidae is a Neotropical family of anurans containing approximately 16 genera and 205 species (Frost 2024). Known colloquially as the poison-dart frogs, dendrobatids are diurnal, lay eggs on land or vegetation, can exhibit bright coloring, and contain powerful neurotoxins, hence their common name. *Leucostethus* Grant, Rada, Anganoy-Criollo, Batista, Dias, Jeckel, Machado, & Rueda-Almonacid, 2017 is a cryptic, forest-dwelling genus of Dendrobatidae with 11 species that range from the rainforests of western Amazonia to the Chocó and Gorgona Island (Vigle et al. 2020; Grant and Bolívar-García 2021).

*Leucostethus bilsa* Vigle, Coloma, Santos, Hernandez-Nieto, Ortega-Andrade, Paluh & Read, 2020 was recently described and is known only from a small area of forest within the 3,000 ha Bilsa Biological Reserve in the Chocó of northwestern Ecuador (Figure 1) (Ortega-Andrade et al. 2010; Vigle et al. 2020). The species is classified as Critically Endangered by the International Union for the Conservation of Nature (IUCN) due to its extremely limited range (a single location) in a highly fragmented habitat (IUCN 2022). The Chocó-Darien Ecoregion has been identified as a top 25 global hotspot for conservation action due to its high levels of biodiversity and endemism, and the rapid rate and extent of habitat loss (Myers et al. 2000; Perlín and Leguizamón 2024). Here we report the discovery of additional *L. bilsa* individuals in the nearby privately protected reserve of the Fundación para la Conservación de los Andes Tropicales (FCAT) and provide an updated distribution map for this species.



Academic editor: Luisa Diele-Viegas

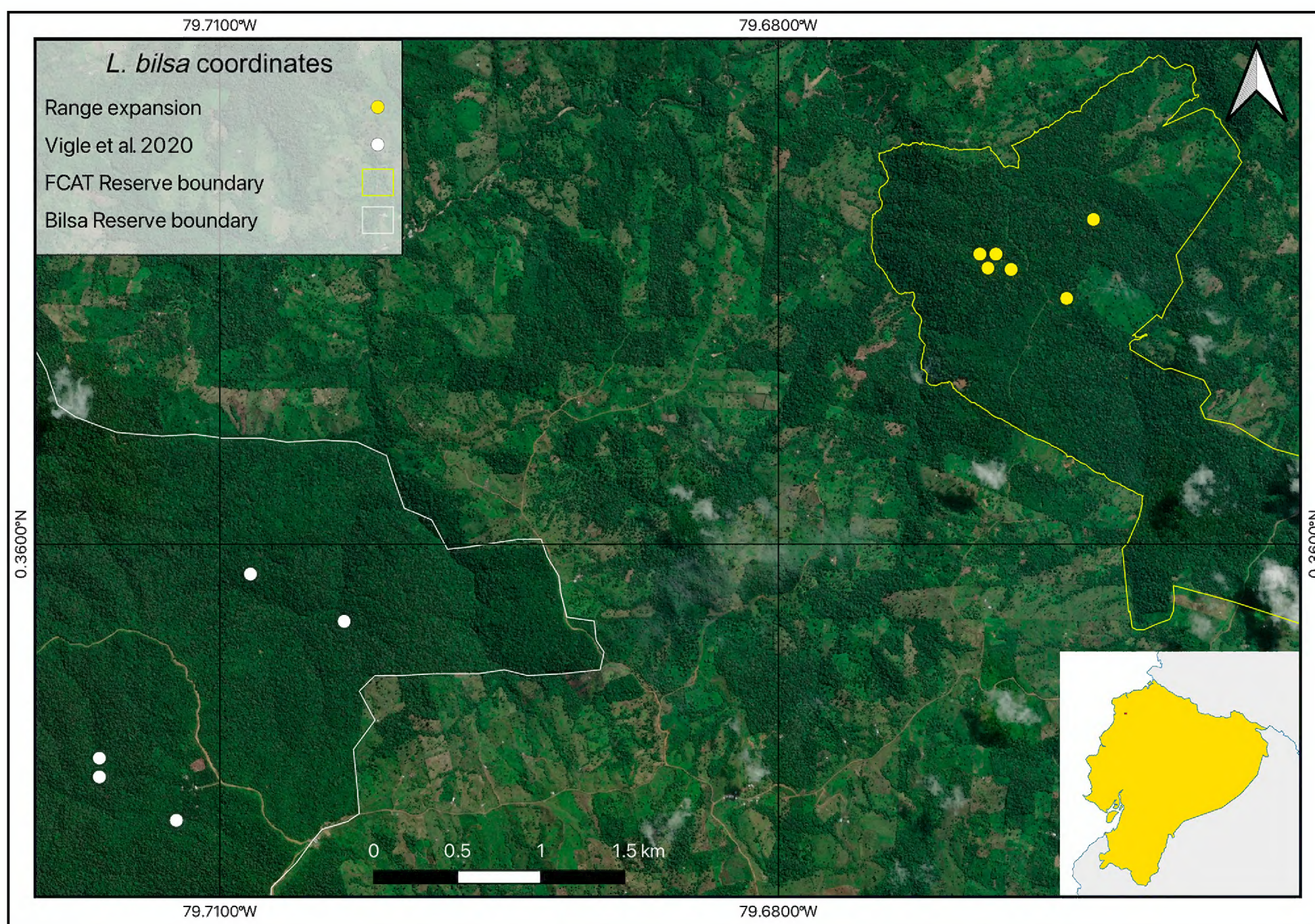
Received: 13 February 2024

Accepted: 26 June 2024

Published: 11 July 2024

Copyright © The authors. This is an open-access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0)





**Figure 1.** Range expansion for the Critically Endangered *Leucostethus bilsa* in the distinct Fundación para la Conservación de los Andes Tropicales (FCAT) Forest Reserve, 5.1 km NE of the Bilsa Biological Reserve, Esmeraldas province, Ecuador.

## METHODS

The FCAT Reserve system encompasses several isolated patches of primary forest totaling 650 ha in the Mache-Chindul mountains between the Cube lagoon (00.3924°N, 079.6473°W, 300 m a.s.l.) and the Bilsa Biological Reserve (00.3555°N, 079.6895°W, 500 m a.s.l.) in Esmeraldas province, Ecuador. The FCAT Reserve primarily comprises tropical rainforests (Jongsma et al. 2014) with an average annual precipitation of 2,500–3,500 mm and average temperature between 23–25.5 °C (Clark et al. 2006). The intervening agricultural mosaic primarily consists of pasture and cacao plantations. Deforestation in the surrounding landscape began in the 1980s and has accelerated in recent years, greatly reducing the number and extent of isolated forest patches between the Bilsa and FCAT Reserves (Perlin and Leguizamón 2024).

We found the first *Leucostethus bilsa* individual incidentally during a visual encounter transect in the core FCAT Reserve as part of a separate seven-month study. The sampling effort comprised 298 survey-hours during the wet and dry seasons of 2022–2023 across 32 sites to compare the amphibian communities of cacao plantations and nearby remnant forests. This first individual was photographed in the field and collected as a voucher specimen. We confirmed the specimen's identification at the Laboratorio de Biología Evolutiva of the Universidad San Francisco de Quito (USFQ), and subsequently deposited the specimen at the Museo de Zoología, USFQ, Quito, Ecuador (ZSFQ). We shared this finding with FCAT workers, who informed us that they had seen similar individuals at various locations within the FCAT Reserve. We asked them to take GPS coordinates and photographs of subsequent *L. bilsa* encounters during their normal field activities throughout the FCAT Reserve using GPS devices and cell phones. We later confirmed these sightings as *L. bilsa*. We note that nobody at FCAT has encountered or heard of *L. bilsa* being found outside of the FCAT or Bilsa Reserves, even though most FCAT employees have lived and worked in the region for decades. In addition, the species has never been reported outside of intact primary forest, despite extensive sampling effort of the amphibian community across the region's agricultural habitats (Mazanec 2022).

We used QGIS v. 3.30.2 (QGIS.org 2024) to map the updated species distribution alongside previously known occurrences as described in Vigle et al. (2020) (Figure 1). We reviewed all available online databases to ensure that *L. bilsa* had not been previously reported from the FCAT Reserve. While additional *L. bilsa* records from the Museo de Zoología at the Pontificia Universidad Católica del Ecuador (QCAZ) exist, we



did not add them to Figure 1 because we were unable to confirm their identity. Similarly, we did not add locations from Ortega-Andrade et al. (2010), who report finding individuals that appear to be *Leucostethus bilsa*, but which they describe as *Colostethus* sp. The authors report finding individuals near the Scientific Station, along the Red, Monkey and Aguacatal trails, and along the Rompefrente and Duchas streams but do not provide precise coordinates for these encounters; as such they were not included in Figure 1 or in our area of occupancy and extent of occurrence calculations. Nevertheless, the locations from both the QCAZ database and Ortega-Andrade et al. (2010) closely align with those reported by Vigle et al. (2020) and are all located within a small area of the Bilsa Biological Reserve.

We used the GeoCAT tool (Bachman et al. 2011) to calculate the species' updated extent of occurrence and area of occupancy following IUCN recommendations (IUCN 2022). Despite using the most recent, available, and cloud-free satellite imagery for our base map (taken in 2017), the imagery overestimates current forest cover in the landscape between the FCAT and Bilsa Reserves. All collection and field research was conducted under permit MAATE-CMARG-2022-0575, issued by the Ministerio del Ambiente, Agua y Transición Ecológica (MAATE) of Ecuador.

## RESULTS

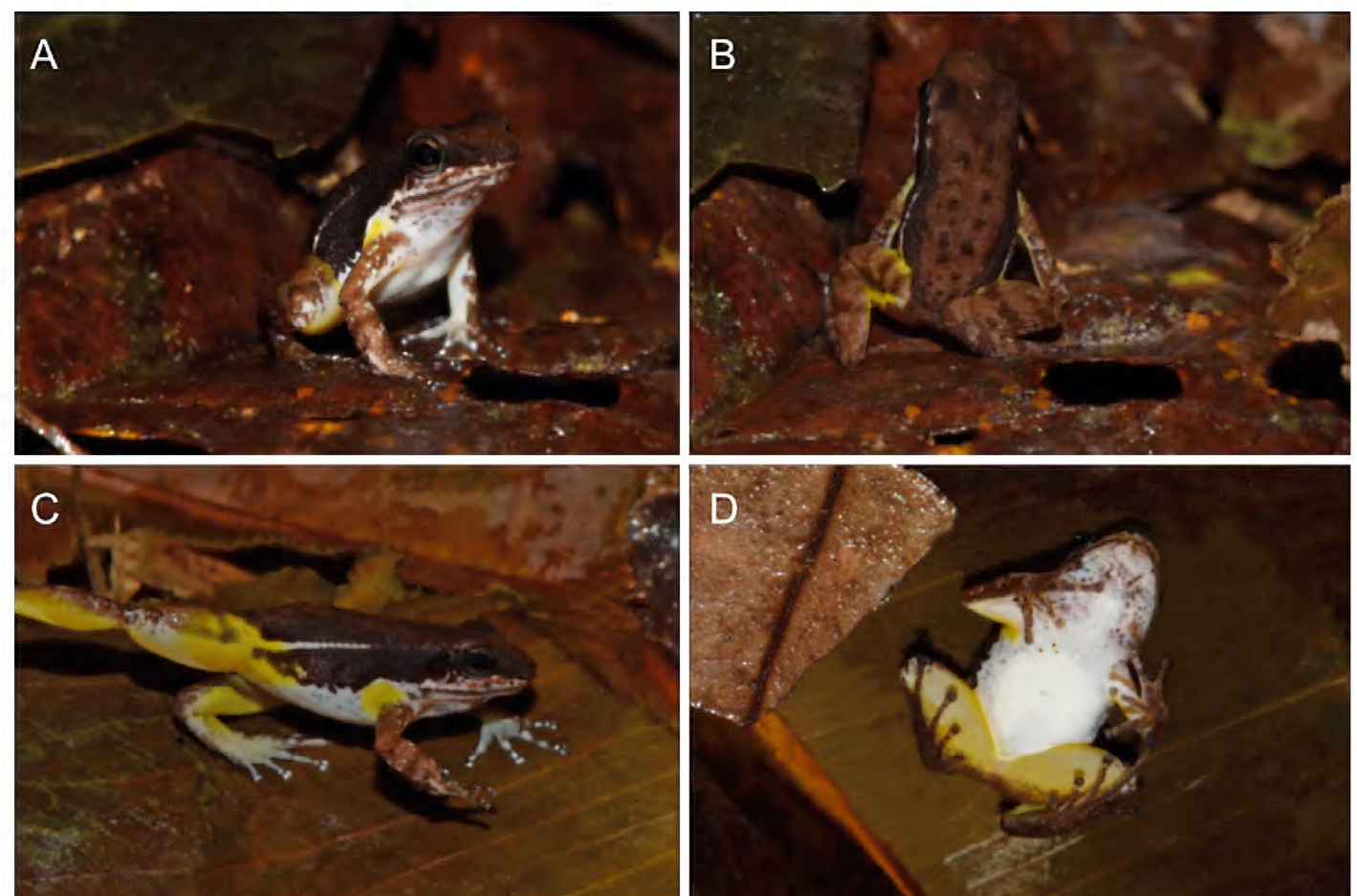
### *Leucostethus bilsa* Vigle, Coloma, Santos, Hernandez-Nieto, Ortega-Andrade, Paluh & Read, 2020

Figure 2

**New records.** ECUADOR – ESMERALDAS • Fundación para la Conservación de los Andes Tropicales (FCAT) Reserve, Guayacán Trail; 00.3748°N, 079.6675°W; 480 m a.s.l.; 8.IX. 2022; S. Holden Jones leg.; 1 sex indet.; found at night (22:30), in leaf litter of mature forest near steep embankment with seasonal stream; ZSFQ 5382 • FCAT Reserve, Guayacán Trail; 00.3754°N, 079.6688°W; 490 m a.s.l.; 15.II. 2023; Darwin Zambrano Cantos obs.; 1 sex indet.; found during day, in leaf litter of mature forest with steep slope • FCAT Reserve, Guayacán Trail; 00.3754°N, 079.6683°W; 490 m a.s.l.; 15.II. 2023; Daniela Franco-Mena obs.; 1 sex indet.; found during day, in leaf litter of mature forest with steep slope • FCAT Reserve, Guayacán Trail; 00.3753°N, 079.6690°W; 480 m a.s.l.; 15.II. 2023; Julio César Loor Vélez obs.; 1 sex indet.; found during day, in leaf litter of mature forest with steep slope • FCAT Reserve, below station; 00.3732°N, 079.6645°W; 450 m a.s.l.; 1.III. 2023; Carlos Aulestia obs.; 1 sex indet.; found during day, next to small seasonal stream • FCAT Reserve, mature forest north of Palacios restoration site; 00.3775°N, 079.6630°W; 500 m a.s.l.; 15.III. 2023; Daniela Franco-Mena obs.; 1 sex indet.; found during day, in leaf litter of mature forest with steep slope (Table 1).

The individuals of *L. bilsa* in the FCAT Reserve appear to represent a distinct IUCN location from those reported by Vigle et al. (2020), Ortega-Andrade et al. (2010), and the QCAZ database due to the independent ownership and management of the FCAT and Bilsa Reserves, and the scarcity of suitable mature forest habitat between them (IUCN 2024). This range expansion increases the species' extent of occurrence from 0.90 to 4.78 km<sup>2</sup>, and the area of occupancy from 8.0 km<sup>2</sup> to 16.0 km<sup>2</sup>. We highlight that despite extensive sampling effort for amphibians in the surrounding agricultural landscape (298 survey-hours, S. Jones et al.

**Figure 2.** *Leucostethus bilsa* individual in the Fundación para la Conservación de los Andes Tropicales (FCAT) Reserve (individual not collected). **A.** Frontal view. **B.** Dorsal view. **C.** Lateral view. **D.** Ventral view. Note the diagnostic cream dorsolateral stripe and mustard-yellow flash coloration. Photos by Daniela Franco-Mena.





**Table 1.** Records of *Leucostethus bilsa* in the Fundación para la Conservación de los Andes Tropicales (FCAT) and Bilsa Biological Reserves as reported in this study and Vigle et al. (2020), including a specimen deposited at the Museo de Zoología, USFQ, Quito, Ecuador (ZSFQ).

No.	Country	Province	Locality	Latitude (°N)	Longitude (°W)	Source/reference
1	Ecuador	Esmeraldas	FCAT Reserve	00.3748	079.6675	ZSFQ 5382, this study
2	Ecuador	Esmeraldas	FCAT Reserve	00.3732	079.6645	Obs., this study
3	Ecuador	Esmeraldas	FCAT Reserve	00.3753	079.6690	Obs., this study
4	Ecuador	Esmeraldas	FCAT Reserve	00.3754	079.6688	Obs., this study
5	Ecuador	Esmeraldas	FCAT Reserve	00.3754	079.6683	Obs., this study
6	Ecuador	Esmeraldas	FCAT Reserve	00.3775	079.6630	Obs., this study
7	Ecuador	Esmeraldas	Bilsa Reserve	00.3583	079.7083	Vigle et al. 2020
8	Ecuador	Esmeraldas	Bilsa Reserve	00.3558	079.7033	Vigle et al. 2020
9	Ecuador	Manabí	Bilsa Reserve	00.3478	079.7164	Vigle et al. 2020
10	Ecuador	Manabí	Bilsa Reserve	00.3451	079.7123	Vigle et al. 2020
11	Ecuador	Manabí	Bilsa Reserve	00.3481	079.7166	Vigle et al. 2020

unpublished), we did not find any additional individuals of *L. bilsa*, suggesting that the species is restricted to mature forest.

**Identification.** All individuals were identified as *L. bilsa* following the original description by Vigle et al. (2020): (i) bright mustard-yellow flash marks in axillae, groin, and anteroventral surfaces of the hindlimbs; (ii) cream oblique dorsolateral stripe; and (iii) white venter (Figure 2). No other species from the lowlands of northwestern Ecuador shares these traits.

DISCUSSION

These new records demonstrate the existence of *Leucostethus bilsa* within the privately protected FCAT Reserve in the Ecuadorian Chocó, which may represent a distinct population separate from the one found at the type locality. Although these populations were likely historically linked, the low degree of remaining forest cover between the FCAT and Bilsa Reserves following decades of deforestation (Perlin and Leguizamón 2024; Figure 1) and the high degree of forest-dependence demonstrated by *L. bilsa* has likely isolated the individuals in the FCAT Reserve from the original population described in Vigle et al. (2020). While these new records increase the species’ extent of occurrence to 4.78 km<sup>2</sup> and area of occupancy to 16.0 km<sup>2</sup>, both metrics still suggest the species is at an elevated risk of extinction. Nevertheless, our discovery of an additional location for the species will likely no longer qualify it as Critically Endangered in future assessments (IUCN 2024). Although a detailed survey of the distribution and demography of *L. bilsa* was outside the scope of this study, we have observed a decline in the area, extent, and quality of potential mature forest habitat between the Bilsa and FCAT Reserves. Future research should seek to address whether *L. bilsa* is present in additional forest fragments in the region and to better understand the species’ population demographics to determine if these populations are isolated or declining.

This finding underscores the importance of conserving the few remaining primary forest patches in the Ecuadorian Chocó for forest-dependent species like *L. bilsa*, as it is likely that additional individuals may be discovered in other smaller forest patches. Additionally, many nearby fragments have been shown to harbor high avian, mammalian, and palm tree diversity (Browne and Karubian 2016; Cook et al. 2020), although amphibians are under sampled in all but the largest patches of forest like the Bilsa and FCAT Reserves (Jongsma et al. 2014).

Matrix composition often has a large effect on the degree of connectivity between fragmented herpetofauna communities which can help sustain seemingly isolated populations (Almeida-Gomes and Rocha 2014; Mendenhall et al. 2014). Even though *L. bilsa* displays a high degree of forest dependence, the abundance of riparian corridors, live fences, and shade-grown cacao in the surrounding landscape provides a framework whereby connectivity may increase following a sufficient degree of forest restoration. Surveying additional forest fragments would help determine the true extent of *L. bilsa*’s range, and better inform conservation and restoration efforts to create a biologically meaningful corridor between the Bilsa and FCAT Reserves, thereby benefiting *L. bilsa* as well as other forest-dependent species.



## ACKNOWLEDGEMENTS

We thank the FCAT team for their assistance throughout the fieldwork portion of this project, and for their work conserving remnant forest in the Ecuadorian Chocó. We would also like to thank Hugo Andrade and an additional anonymous reviewer whose feedback improved the manuscript.

## ADDITIONAL INFORMATION

### Conflict of interest

The authors declare that no competing interests exist.

### Ethical statement

No ethical statement is reported.

### Funding


This work was financially supported by Fulbright Ecuador, The Rufford Foundation, and the ARCS Foundation Honolulu Chapter.


### Author contributions


Conceptualization: SHJ, DFM, RAZ, JMG. Data curation: SHJ, DFM. Formal analysis: SHJ,. Funding acquisition: SHJ, RAZ, JMG. Investigation: SHJ, DFM, DZC, JCLV, CA, RAZ, JMG. Methodology: SHJ, DFM, RAZ, JMG. Resources: JMG. Supervision: RAZ, JMG. Visualization: SHJ, DFM. Project administration: SHJ, JMG. Software: SHJ. Validation: JMG, DFM. Writing – original draft: SHJ. Writing – review and editing: SHJ, DFM, RAZ, JMG.

### Author ORCID iDs

S. Holden Jones  <https://orcid.org/0000-0001-5790-5175>

Daniela Franco-Mena  <https://orcid.org/0000-0003-3655-2678>

Rakan A. Zahawi  <https://orcid.org/0000-0002-5678-2967>

Juan M. Guayasamin  <https://orcid.org/0000-0003-0098-978X>

### Data availability

All data that support the findings of this study are available in the main text and Supplementary materials.

### Supplementary file

**Table S1.** All known localities for *Leucostethus bilsa*. Location data used in preparing Figure 1.

## REFERENCES

- Almeida-Gomes M, Rocha CFD** (2014) Landscape connectivity may explain anuran species distribution in an Atlantic forest fragmented area. *Landscape Ecology* 29 (1): 29–40. <https://doi.org/10.1007/s10980-013-9898-5>
- Bachman S, Moat J, Hill AW, de la Torre J, Scott B** (2011) Supporting Red List threat assessments with GeoCAT: geo-spatial conservation assessment tool. In: Smith V, Penev L (Eds) e-Infrastructures for data publishing in biodiversity science. *ZooKeys* 150: 117–126. <https://doi.org/10.3897/zookeys.150.2109>
- Browne L, Karubian J** (2016) Diversity of palm communities at different spatial scales in a recently fragmented tropical landscape. *Botanical Journal of the Linnean Society* 182 (2): 451–464. <https://doi.org/10.1111/boj.12384>
- Clark JL, Neil DA, Asanza M** (2006) Floristic checklist of the Mache-Chindul Mountains of northwestern Ecuador. *Contributions from the United States National Herbarium* 54: 1–180.
- Cook RN, Ramirez-Parada T, Browne L, Ellis M, Karubian J** (2020) Environmental correlates of richness, community composition, and functional traits of terrestrial birds and mammals in a fragmented tropical landscape. *Landscape Ecology* 35 (12): 2825–2841. <https://doi.org/10.1007/s10980-020-01123-4>
- Frost DR** (2024) Amphibian species of the world: an online reference. <https://amphibiansoftheworld.amnh.org/index.php>. American Museum of Natural History, New York, USA. Accessed on: 2024-1-3.
- Grant T, Bolívar-García W** (2021) A new species of *Leucostethus* (Anura, Dendrobatidae) from Gorgona Island, Colombia. *ZooKeys* 1057: 185–208. <https://doi.org/10.3897/zookeys.1057.67621>
- Grant T, Rada M, Anganoy-Criollo M, Batista A, Dias PH, Jeckel AM, Machado DJ, Rueda-Almonacid JV** (2017) Phylogenetic systematics of dart-poison frogs and their relatives revisited (Anura: Dendrobatoidea). *South American Journal of Herpetology* 12 (s1): S1–S90. <https://doi.org/10.2994/sajh-d-17-00017.1>
- IUCN SSC Amphibian Specialist Group** (2022) *Leucostethus bilsa*. The IUCN Red List of Threatened Species 2022: e.T195586657A195586662. Accessed on: 2024-1-25.
- IUCN Standards and Petitions Committee** (2024) Guidelines for Using the IUCN Red List categories and criteria. Version 16. IUCN Standards and Petitions Committee. [https://nc.iucnredlist.org/redlist/content/attachment\\_files/RedListGuidelines.pdf](https://nc.iucnredlist.org/redlist/content/attachment_files/RedListGuidelines.pdf). Accessed on: 2024-6-22.

- Jongsma GFM, Hedley RW, Durães R, Karubian J** (2014). Amphibian diversity and species composition in relation to habitat type and alteration in the Mache-Chindul Reserve, northwest Ecuador. *Herpetologica* 70 (1): 34–46. <https://doi.org/10.1655/herpetologica-d-12-00068>
- Mazanec T** (2022) Herpetofauna richness, community composition and abundance in forested and agricultural habitats of the Mache Chindul Reserve, Northwest Ecuador. Masters thesis, Tulane University, New Orleans, USA, 30 pp.
- Mendenhall CD, Frishkoff LO, Santos-Barrera G, Pacheco J, Mesfun E, Quijano FM, Ehrlich PR, Ceballos G, Daily GC, Pringle RM** (2014) Countryside biogeography of Neotropical reptiles and amphibians. *Ecology* 95 (4): 856–870. <https://doi.org/10.1890/12-2017.1>
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J** (2000) Biodiversity hotspots for conservation priorities. *Nature* 403 (6772): 853–858. <https://doi.org/10.1038/35002501>
- Ortega-Andrade HM, Bermingham J, Aulestia C, Paucar C** (2010) Herpetofauna of the Bilsa Biological Station, province of Esmeraldas, Ecuador. *Check List* 6 (1): 119. <https://doi.org/10.15560/6.1.119>
- Perlin L, Leguizamón A** (2024) Agriculture-driven deforestation in Ecuador’s Mache-Chindul Ecological Reserve: the farmers’ perspective. *Journal of Rural Studies* 107: 103263. <https://doi.org/10.1016/j.jrurstud.2024.103263>
- QGIS.org** (2024) QGIS Geographic Information System. QGIS Association. <http://qgis.org>. Accessed on: 2024-1-25.
- Ron SR** (2018) Base de datos de la colección de anfibios del Museo de Zoología QCAZ. Pontificia Universidad Católica del Ecuador. <https://bioweb.bio/portal/>. Accessed on: 2024-1-31.
- Vigle GO, Coloma LA, Santos JC, Hernandez-Nieto S, Ortega-Andrade HM, Paluh DJ, Read M** (2020) A new species of *Leucostethus* (Anura: Dendrobatidae) from the Cordillera Mache-Chindul in northwestern Ecuador, with comments on similar *Colostethus* and *Hyloxalus*. *Zootaxa* 4896 (3): 342–372. <https://doi.org/10.11646/zootaxa.4896.3.2>